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Propofol suppresses invasion and induces apoptosis of osteosarcoma cell *in vitro* via downregulation of TGF-β1 expression

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Abstract. – OBJECTIVE: Osteosarcoma (OS) is the most common malignant tumor of the bone, with a high mortality rate and poor prognosis. Propofol has been proposed to play a role of antitumor in various cancers. However, the functions and mechanisms of propofol in OS is still not clear.

MATERIALS AND METHODS: The different concentrations of propofol were co-incubated with osteosarcoma MG-63 lines for 72 hrs. Cell proliferation, apoptosis, and invasion were detected by MTT assay, Flow cytometry analysis, and Matrigel invasion assay. Western blot was used to detect the TGF-β1 protein levels. MG-63 cells were treated with human recombinant TGF-β1 (rh TGF-β1) to assess the role of TGF-β1 in propofol-induced anti-tumor activity.

RESULTS: Propofol significantly inhibited cell proliferation and invasion and promoted apoptosis of MG-63 lines cells. Propofol also efficiently reduced TGF-β1 expression. Moreover, restoration of TGF-β1 by rhTGF-β1 treatment reversed the effects of propofol on the biological behavior of OS cells.

CONCLUSIONS: Propofol can effectively inhibit proliferation and invasion and induce apoptosis of OS cells through, at least partly, downregulation of TGF-β1 expression.

Key Words:
Osteosarcoma, Propofol, TGF-β1, Metastasis, Apoptosis.

Introduction

Osteosarcoma (OS) is the most common of all bone malignancies, affecting predominantly children, teenagers, and young adults aged 10-30 year[1]. The biology feature of OS is high propensity for 90% pulmonary metastasis[2]. However, the molecular mechanisms underlying the carcinogenesis, progression, and aggressiveness of the cancer have not been fully elucidated.

Transforming growth factor (TGF)-β is involved in physiologic processes, such as wound healing, tissue development and remodeling. TGF-β has also been implicated in many pathologic conditions, including cancer, and has been shown to regulate some events such as angiogenesis, immune suppression, and cell migration[3-5]. Transforming growth factor-β promotes cancer metastasis through various mechanisms including immunosuppression, angiogenesis, and invasion. In patients with osteosarcoma, serum level of TGF-β was significantly increased, which was positive for distant metastasis[6]. It was indicated that TGF-β may be closely involved in the pathogenesis of osteosarcoma. Experimental studies have found TGF-β overexpression promoted invasion and growth of cancer cells, and vice versa[7-10]. In addition, combining dendritic cells with anti-TGF-β antibody enhanced the systemic immune response[11].

Propofol (2, 6-diisopropylphenol) is an intravenous sedative-hypnotic agent administered to induce and maintain anesthesia. It has been recently revealed that propofol has anticancer properties including direct and indirect suppression of the viability and proliferation of cancer cells by promoting apoptosis in some cancer cell lines[12-14]. We have previous reported that propofol could promote invasion and proliferation of ovarian cancer, pancreatic cancer and esophageal squamous cell carcinoma cell by Slug, S100a4, NF-kB and ERK-VEGF/MMP-9 signals, respectively[15-18]. Therefore, propofol might be a better agent than other anesthetics for cancer surgery[19].

Although propofol induces apoptosis and inhibits the invasion of cancer cells both *in vitro* and

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in vivo by different molecular mechanisms[15-18], we focused on the anti-cancer properties of propofol that are regulated via TGF-β1 signaling pathway, which regulates the different molecular above[20-22]. The aim of this study is to investigate the effects of propofol on the biological behavior of human OS cells and its related molecular mechanisms.

Materials and Methods

Cell Culture

The human osteosarcoma cell lines MG-63 were gifted by Ma et al[23], and cultured as conventional methods. The cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 with 10% fetal calf serum (FCS, Gibco, Grand Island, NJ, USA), at 37°C in 5% carbon dioxide and 95% air. In all of the assays, a monolayer of cells that was 50-70% confluent was used. All the methods used were according to the manufacture's instruction.

Cell Viability Assay

MG-63 cells were cultured in 96-well plates (3×10^4 per well) and treated with 10 μg/mL propofol for 72 hs. To determine the signaling pathways involved in the production of TGFβ1, MG-63 cells were preincubated with rh TGF-β1 (5 ng/mL) (Cell Signaling Technology, Guangzhou, China) or DMSO (controls) 6h before the addition of propofol. Determination of viable cells was performed by adding 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H- tetrazolium bromide (MTT; Sigma-Aldrich, St. Louis, MO, USA) to a final concentration of 0.5 mg/ml. After 3 hours incubation at 37°C and 5% CO_2 in a humidified incubator, the formazan crystals were resolved by 10% SDS in 10 mM HCl and the absorption was measured at 560 nm. MTT measurements were performed daily for 3 consecutive days, as triplicates in three independent experiments. The absorption data were used to calculate the doubling time of each cell line.

Apoptosis Assay

After treatment above, MG-63cells were collected and fixed overnight at 4 °C with 75% ethanol for propidium iodide staining and Flow cytometry analysis was used to evaluate sub-G1 cell populations (Apoptotic rate).

Matrigel Invasion Assay

The invasiveness of MG-63 cells was tested after treatment as described above. The cells (1×10^6/mL) were added to the upper wells coated with Matrigel with serum-free medium containing 25 μg/mL fibronectin as a chemoattractive agent in the lower wells. After a 24hs incubation period, the number of cells on the lower side of the membrane was counted in five random fields after staining with Hema-3 kit.

Western Blot Analysis

Cells were cultured, pelleted by centrifugation, washed with ice-cold phosphate buffered saline (PBS), and lysed with radioimmunoprecipitation assay (RIPA) buffer supplemented with a protease inhibitor. TGFβ1 was detected as the methods depicted before[23]. Anti-TGF-β1 (1:1000) was purchased from Santa Cruz Biotechnology (Shanghai, China). β-Actin was obtained from Cell Signaling Technology (Shanghai, China). Blots were visualized by using ECL Plus Western Blotting Detection Reagents according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean ± SD. SPSS 11.0 software (SPSS Inc., Chicago, IL, USA) was used for analysis. The difference of statistical significance between two groups was assessed using Student's *t*-tests (one-tailed). $p < 0.05$ was considered to indicate a statistically significant result. All experiments were repeated at least three times.

Results

Effect of Propofol on TGF-β1 Expression in MG-63cells

MG-63 and U-2OS cells were treated with propofol (0-10 μg/mL) for 72 hs. Western blot analysis was used to detect the TGF-β1 protein level after treatment. Cells treated with propofol displayed a dose-dependent reduction in the expression levels of TGF-β1 protein. These data confirmed the suppression effect of propofol and established the efficiency of propofol treatment.

Effect of Propofol on Cell Survival and Apoptosis

MG-63cells were treated with propofol (10 μg/mL) for 72 hs. The analysis of cell survival using the MTT assay showed a significantly decrease in cell survival for propofol-treated cells when compared with the control cells (Figure 2A).

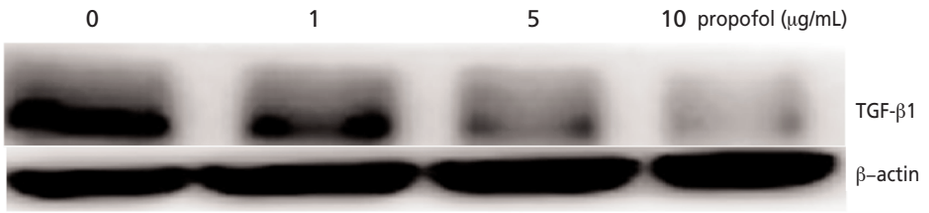

Figure 1. TGF-β1 gene knockdown by propofol treatment in MG-63 cells. MG-63 cells were treated with propofol (0-10 μg/mL) for 72 hs. Representative images showing expression of TGF-β1 protein in both of the cells as analyzed by Western blot. β-Actin was as a control.

Cell apoptosis was determined using propidium iodine (PI) staining followed by flow cytometry analysis. Cells treated with propofol (10 μg/mL) for 72 hs showed significant apoptosis, suggesting propofol-induced cell apoptosis (Figure 2B).

Effect of Propofol on Invasive Capability of MG-63 Cells

In vitro studies were done to determine the effects of propofol on invasive capability of MG-63 cells. The invasion of MG-63 cells was markedly inhibited, compared with control cells ($p < 0.05$) (Figure 3A). The migration of MG-63 cells was also markedly inhibited compared with control cells ($p < 0.05$) (Figure 3B). These results suggest that propofol inhibits cell migration and invasion.

Propofol Inhibits cell Survival and Induces Apoptosis by TGF-β1 Inhibition

MG-63 cells were preincubated with rh TGF-β1 (10 ng/mL) or DMSO (controls) 6h before the addition of propofol. The findings showed that although propofol inhibited cell survival and induced apoptosis, when treated with rhTGF-β1, the cell survival was increased and the apoptosis was inhibited (Figure 2).

Propofol Inhibits Migration and Invasion by TGF-β1 Inhibition

MG-63 cells were preincubated with rh TGF-β1 (10 ng/mL) or dimethyl sulfoxide (DMSO) (controls) 6h before the addition of propofol. The results showed that rh TGF-β1 restored the

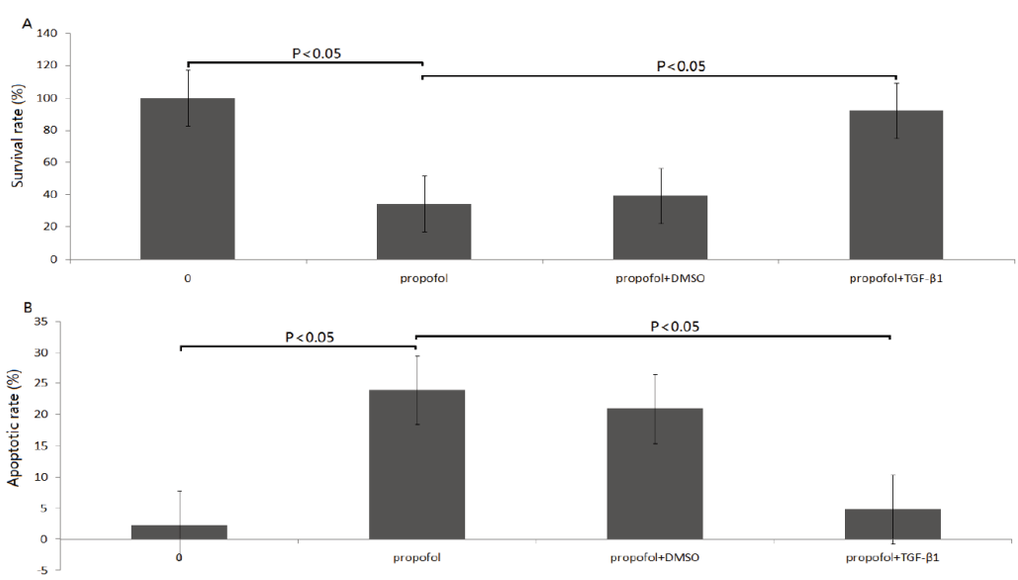

Figure 2. Effect of propofol on cell survival and apoptosis. ***A,*** MG-63 cells were treated with propofol (10 μg/mL) for 72 hs, cell survival rate was detected by MTT assay. ***B,*** MG-63 cells were treated with propofol (10 μg/mL) for 72 hs, cell apoptotic rate was detected by flow cytometry assay.

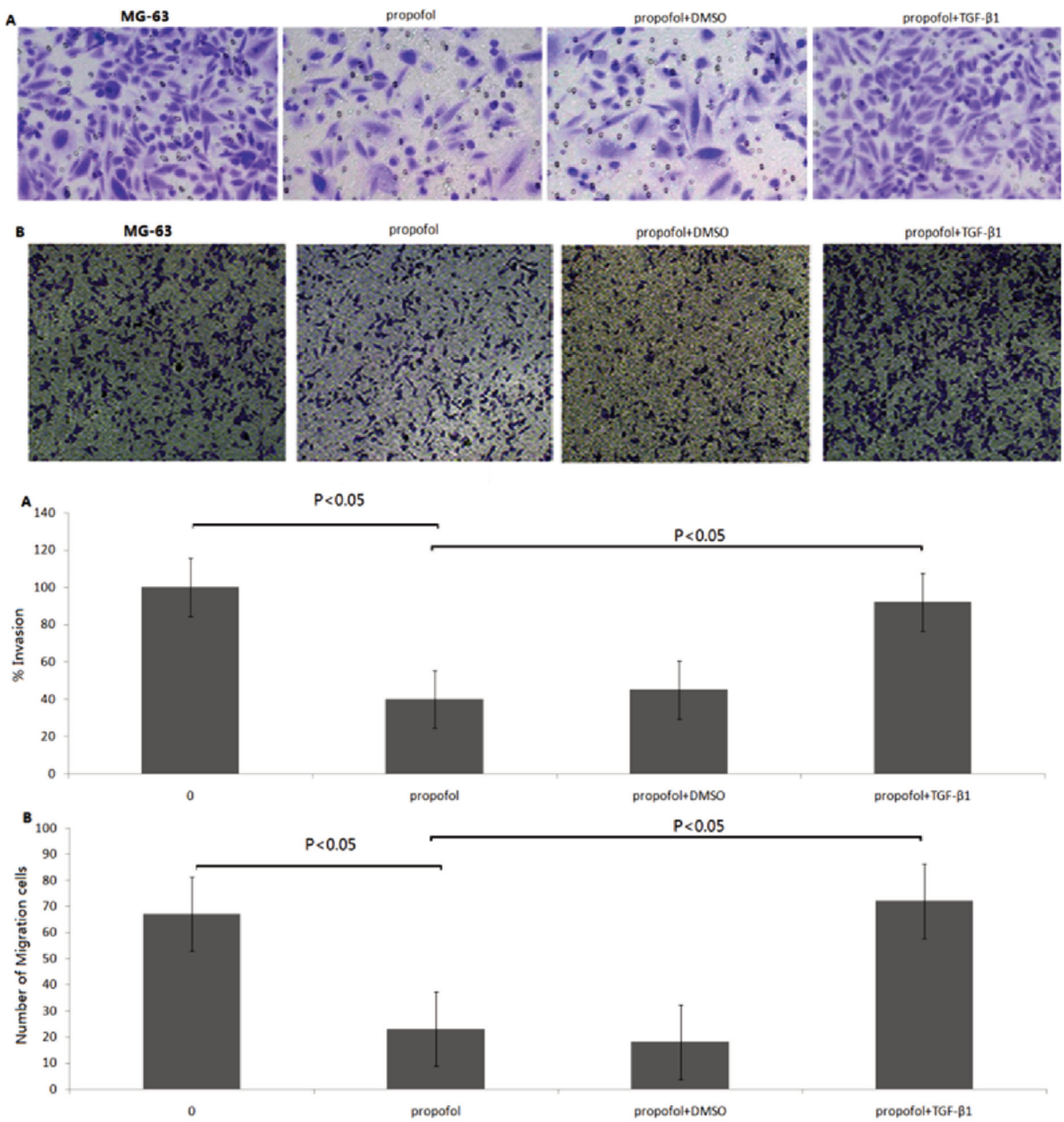

Figure 3. Effect of propofol on invasive capability of MG-63 cells. ***A,*** MG-63 cells were detected by Transwell invasion assay. ***B,*** The migration of MG-63 cells were detected by Matrigel invasion assay. $^{*}p < 0.05$ compared with respective controls.

migration and invasion of the MG-63/propofol cells (Figure 3A-3B).

Discussion

Osteosarcoma (OS) is the most common malignant tumor in bones. Metastasis is a major cause of mortality and morbidity[24]. Although the five-year survival rate has improved to ~60% for patients without metastasis, the prognosis remains poor for patients with metastatic OS. The rarity of OS coupled with the challenges of drug development for metastatic cancers have slowed the delivery of improvements in long-term outcomes for these patients[25]. In this study, we evaluated the effects of propofol on the behavior of human OS cells and found that propofol inhibited MG-63 cell proliferation and invasion, and induced apoptosis. The results were consistent with our previ-

ous findings and those of other studies[13-18]. This evidence suggests that propofol may be as an anesthetic particularly suitable for the peri-operative phase in cancer surgery.

To clarify the mechanism involved in the suppression of MG-63 cells, the effect of propofol on TGF-β1 expression was examined. Overexpression of TGF-β1 has been implicated in promoting immune suppression, tumor angiogenesis, tumor cell migration, and invasion in many cancers, including OS[26]. The targeted down-regulation of TGF-β1 expression inhibited growth and invasion in OS and many other cancer cells[27-30].

In the present study, we found that propofol treatment significantly decreased TGF-β1 expression in a dose-dependent manner. Also, decreased TGF-β1 expression results in the induction of cell apoptosis and the inhibition of cell invasion, indicating that TGF-β1 acts as a tumor-promotor. More importantly, restoration of TGF-β1 by rhTGF-β1 treatment reversed the effects of propofol in MG-63 cells. These results suggested that the anti-tumor effect of propofol might be owing to the downregulation of TGF-β1. However, the detailed mechanisms of how propofol influences TGF-β1 expression are still unclear, and further clarification is needed in the future.

Conclusions

Propofol could inhibit proliferation and invasion, and induce apoptosis of OS cells. The modulation of TGF-β1 may contribute to these anti-tumor actions. Further studies are needed to validate the clinical relevance of propofol.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Broadhead ML, Clark JC, Myers DE, Dass CR, Choong PF. The molecular pathogenesis of osteosarcoma: a review. Sarcoma 2011; 2011: 959248.
2) Jaffe N. Osteosarcoma: review of the past, impact on the future. The American experience. Cancer Treat Res 2009; 152: 239-262.
3) Gotzmann J, Mikula M, Eger A, Schulte-Hermann R, Foisner R, Beug H, Mikulits W. Molecular aspects of epithelial cell plasticity: implications for local tumor invasion and metastasis. Mutat Res 2014; 566: 9-20.
4) Bierie B, Moses HL. TGF-beta and cancer. Cytokine Growth Factor Rev 2006; 17: 29-40.
5) Jakowlew SB. Transforming growth factor-beta in cancer and metastasis. Cancer Metastasis Rev 2006; 25: 435-457.
6) Xu S, Yang S, Sun G, Huang W, Zhang Y. Transforming growth factor-beta polymorphisms and serum level in the development of osteosarcoma. DNA Cell Biol 2014; 33: 802-806.
7) Li F, Li S, Cheng T. TGF-β1 promotes osteosarcoma cell migration and invasion through the miR-143-versican pathway. Cell Physiol Biochem 2014; 34: 2169-2179.
8) Chen J, Song Y, Yang J, Gong L, Zhao P, Zhang Y, Su H. The up-regulation of cysteine-rich protein 61 induced by transforming growth factor beta enhances osteosarcoma cell migration. Mol Cell Biochem 2013; 384: 269-277.
9) Klotzsche-von Ameln A, Muschter A, Mamlouk S, Kalucka J, Prade I, Franke K, Rezaei M, Poitz DM, Breier G, Wielockx B. Inhibition of HIF prolyl hydroxylase-2 blocks tumor growth in mice through the antiproliferative activity of TGFβ. Cancer Res 2011; 71: 3306-3316.
10) Tu B, Peng ZX, Fan QM, Du L, Yan W, Tang TT. Osteosarcoma cells promote the production of pro-tumor cytokines in mesenchymal stem cells by inhibiting their osteogenic differentiation through the TGF-β/Smad2/3 pathway. Exp Cell Res 2014; 320: 164-173.
11) Kawano M, Itonaga I, Iwasaki T, Tsuchiya H, Tsumura H. Anti-TGF-β antibody combined with dendritic cells produce antitumor effects in osteosarcoma. Clin Orthop Relat Res 2012; 470: 2288-2294.
12) Xu YJ, Li SY, Cheng Q, Chen WK, Wang SL, Ren Y, Miao CH. Effects of anaesthesia on proliferation, invasion and apoptosis of LoVo colon cancer cells in vitro. Anaesthesia 2016; 71: 147-154.
13) Wang ZT, Gong HY, Zheng F, Liu DJ, Yue XQ. Propofol suppresses proliferation and invasion of gastric cancer cells via downregulation of microRNA-221 expression. Genet Mol Res 2015; 14: 8117-8124.
14) Zhang L, Wang N, Zhou S, Ye W, Jing G, Zhang M. Propofol induces proliferation and invasion of gallbladder cancer cells through activation of Nrf2. J Exp Clin Cancer Res 2012; 31: 66.
15) Wang P, Chen J, Mu LH, Du QH, Niu XH, Zhang MY. Propofol inhibits invasion and enhances paclitaxel- induced apoptosis in ovarian cancer cells through the suppression of the transcription factor slug. Eur Rev Med Pharmacol Sci 2013; 17: 1722-1729.

16) Guo XG, Wang S, Xu YB, Zhuang J. Propofol suppresses invasion, angiogenesis and survival of EC-1 cells in vitro by regulation of S100A4 expression. Eur Rev Med Pharmacol Sci 2015; 19: 4858-4865.

17) Du QH, Xu YB, Zhang MY, Yun P, He CY. Propofol induces apoptosis and increases gemcitabine sensitivity in pancreatic cancer cells in vitro by inhibition of nuclear factor-kB activity. World J Gastroenterol 2013; 19: 5485-5492.

18) Xu YB, Du QH, Zhang MY, Yun P, He CY. Propofol suppresses proliferation, invasion and angiogenesis by down-regulating ERK-VEGF/MMP-9 signaling in Eca-109 esophageal squamous cell carcinoma cells. Eur Rev Med Pharmacol Sci 2013; 17: 2486-2494.

19) Inada T, Kubo K, Shingu K. Possible link between cyclooxygenase-inhibiting and antitumor properties of propofol. J Anesth 2011; 25: 569-575.

20) Jin L, Zhu C, Wang X, Li C, Cao C, Yuan J, Li S. Urocortin attenuates TGFβ1-induced Snail1 and slug expressions: inhibitory role of Smad7 in Smad2/3 signaling in breast cancer cells. J Cell Biochem 2015; 116: 2494-2503.

20) Stanisavljevic J, Porta-de-la-Riva M, Batlle R, de Herreros AG, Baulida J. The p65 subunit of NF-κB and PARP1 assist Snail1 in activating fibronectin transcription. J Cell Sci 2011; 124: 4161-4171.

21) Cheng S, Guo J, Yang Q, Han L. Crk-like adapter protein is required for TGF-β-induced AKT and ERK-signaling pathway in epithelial ovarian carcinomas. Tumour Biol 2015; 36: 915-919.

22) Jin L, Zhu C, Wang X, Li C, Cao C, Yuan J, Li S. S100A4 mediates endometrial cancer invasion and is a target of TGF-beta1 signaling. Lab Invest 2009; 89: 937-947.

23) Ma JF, Liu L, Yang WJ, Zang LN, Xi YM. RNAi-mediated knockdown of relaxin decreases in vitro proliferation and invasiveness of osteosarcoma MG-63 cells by inhibition of MMP-9. Eur Rev Med Pharmacol Sci 2013; 17: 1102-1109.

24) Ren XF, Zhao H, Gong XC, Wang LN, Ma JF. RLN2 regulates in vitro invasion and viability of osteosarcoma MG-63 cells via S100A4/MMP-9 signal. Eur Rev Med Pharmacol Sci 2015; 19: 1030-1036.

25) Barbato S, Sgarbi G, Gorini G, Baracca A, Solaini G. The inhibitor protein (IF1) of the F1F0-ATPase modulates human osteosarcoma cell bioenergetics. J Biol Chem 2005; 290: 6338-6348.

26) Jung ST, Moon ES, Seo HY, Kim JS, Kim GJ, Kim YK. Expression and significance of TGF-beta isoform and VEGF in osteosarcoma. Orthopedics 2005; 28: 755-760.

27) Niu G, Li B, Sun L, An C. MicroRNA-153 inhibits osteosarcoma cells proliferation and invasion by targeting TGF-β2. PLoS One 2015; 10: e0119225.

28) Ren XF, Mu LP, Jiang YS, Wang L, Ma JF. LY2109761 inhibits metastasis and enhances chemosensitivity in osteosarcoma MG-63 cells. Eur Rev Med Pharmacol Sci 2015; 19: 1182-1190.

29) Wang XG, Meng Q, Qi FM, Yang QF. Blocking TGF-β inhibits breast cancer cell invasiveness via ERK/S100A4 signal. Eur Rev Med Pharmacol Sci 2014; 18: 3844-3853.

30) Kwak HJ, Park MJ, Cho H, Park CM, Moon SI, Lee HC, Park IC, Kim MS, Rhee CH, Hong SI. Transforming growth factor-beta1 induces tissue inhibitor of metalloproteinase-1 expression via activation of extracellular signal-regulated kinase and Sp1 in human fibrosarcoma cells. Mol Cancer Res 2006; 4: 209-220.